EXHIBIT J

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Tumor Incidence in a Chemical Carcinogenesis Study of Nonhuman Primates

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This report covers a 32-year period of an ongoing chemical carcinogenesis study in nonhuman primates, which was initiated by the National Cancer Institute in 1961. Autopsy records of 373 breeders and normal controls showed very low incidence of spontaneous malignant tumors in cynomolgus (1.5%) and rhesus (2.8%) monkeys, but considerably higher incidence in African green monkeys (8%). A large number of substances including a variety of food additives, food components, environmental contaminants, *N*-nitroso compounds, "classical" rodent carcinogens, antineoplastic agents, and immunosuppressive agents have been evaluated for long-term carcinogenic activity. Food components tested which are probably most relevant to human exposure are the artificial sweeteners, cyclamate and saccharin. After 22 years of continuous dosing, neither cyclamate nor saccharin have shown any evidence of carcinogenic effects. Similarly, the tumorigenic potential of arsenic and DDT was negligible after dosing for 15–22 years. In contrast, the fungal food contaminants, aflatoxin B_1 (AFB_1) and sterigmatocystin (SMT), were found to be potent hepatocarcinogens. AFB_1 also induced adenocarcinomas of the pancreas, osteosarcomas, and other tumors. Also, the aglycone of cycasin, MAM acetate, induced a variety of tumors, but primarily hepatocellular and renal cell carcinomas. The compounds most recently introduced into the colony include three heterocyclic amines present in cooked meat. One of these compounds, 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) has proven to be one of the most potent hepatocarcinogens in the history of the monkey project, inducing malignant liver tumors in 65% of animals over a 7-year period of exposure. Of the classical rodent carcinogens studied, urethane was the only one which produced malignant tumors in the monkeys. Conversely, all except two of the *N*-nitroso compounds were carcinogenic. Diethylnitrosamine (DENA) was the most potent and predictable hepatocarcinogen in cynomolgus, rhesus, and African green monkeys. However, when administered intraperitoneally to galagos (a prosimian), DENA induced primarily mucoepidermoid carcinoma of the nasal cavity. *N*-Methyl-*N*-nitrosourea (MNU) was the only carcinogen persistently producing tumors in the digestive tract, mostly squamous cell carcinomas of the esophagus. Among the antineoplastic and immunosuppressive agents, procarbazine (MIH) was the only unequivocal carcinogen, with a 33% tumor incidence, causing acute nonlymphocytic leukemia in most of the cases.

INTRODUCTION

The induction of cancer by chemicals involves a complex series of sequences that convert a normal to a neoplastic cell (Williams and Weisburger, 1991). Extrapolation

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of human carcinogenic risk has traditionally been based on rodent data (Iatropoulos, 1992; Gold *et al.*, 1991), since it is not practical to use larger animals, such as nonhuman primates, for large-scale carcinogenesis bioassays. Nevertheless, nonhuman primates represent a valuable model for predicting human carcinogenic risk, due to their close phylogenetic relationship to humans, low spontaneous tumor rate, relative longevity, and large organ size. Earlier studies on drug metabolism demonstrated similarities between man and monkeys (Smith and Williams, 1974), but there has been paucity of more recent reports on the characterization of metabolic pathways in monkeys. Studies on metabolic activation of heterocyclic aromatic amines (HAA) in cynomolgus monkeys vs rat and man have revealed species differences in the hepatic P450-mediated metabolic activation of HAA among rats, monkeys, and humans (Davis *et al.*, 1993a, 1993b).

This project represents the largest and longest chemical carcinogenesis study in nonhuman primates ever undertaken in this country. Old World monkeys were used to evaluate a large number of substances, some of which have been administered for many years (Adamson and Sieber, 1983). The primary reason for initiating this study in 1961 was the lack of information on the susceptibility of nonhuman primates to chemicals known to produce tumors in rodents. More recently, one of the prime objectives was to evaluate the long-term effects of potential human carcinogens, which are consumed in food, are present in the environment, or are used as chemotherapeutic agents. The data on spontaneous and chemically induced tumor incidence since the onset of the study in 1961 are presented in this report.

METHODS

The monkey colony currently consists of three species: *Macaca fascicularis* (cynomolgus), *Macaca mulatta* (rhesus), and *Cercopithecus aethiops* (African green). All three species were bred since the onset of the program in 1961, and served as the primary source for experimental animals in this study. However, in addition to the breeders, a small number of the experimental animals used in this project were feral monkeys, i.e., for studies on arsenic and sterigmatocystin. For the past 7 years, only cynomolgus monkeys have been bred due to their smaller size and very low spontaneous tumor rate. Presently, the colony consists of 239 cynomolgus, 98 rhesus, and 18 African green monkeys, which includes a breeding colony of 33 animals. Between 20 and 30 babies are born each year. For over 20 years, infants were separated from their mothers at birth and hand reared, but currently newborns stay with their mothers until they are 5–6 months old, and are then weaned. The diet consists of High Protein Purina Monkey Chow (5045 Standard), vitamin mixture spread on sandwiches, and apples. The monkeys are housed individually in stainless steel cages, which provide appropriate space as required by the Animal Welfare Act.

Since the initiation of this chemical carcinogenesis study, a variety of substances have been tested (Table 1). The compounds were generally administered orally (po), in a vitamin mixture on a sandwich, by nasogastric intubation (ng), intraperitoneally (ip), or intravenously (iv). At least five monkeys were assigned to each control group that received only the vehicle for the different compounds, including dimethyl sulfoxide (DMSO), hydroxypropyl cellulose (HPC), corn oil, olive oil, saline, distilled water, vitamin spread, syrup, and apple sauce (see Table 1). Periodically, monkeys were also

TABLE 1

SUBSTANCES TESTED FOR CARCINOGENIC ACTIVITY IN NONHUMAN PRIMATES

Common name or chemical name	Route of administration	Vehicle used
Therapeutic agents		
Procarbazine (MIH)	sc, ip, po	Sterile H_2O
Adriamycin (doxorubicin)	iv	Sterile saline
Melphalan (L-PAM)	ng	DMSO
Azathioprine (Imuran)	po	HPC
Cyclophosphamide (Cytoxan)	po	Distilled H_2O
Food additives, food components, and environmental contaminants		
Aflatoxin B_1 (AFB_1)	po, ip	DMSO
MAM acetate[a] (cycasin)	po	Sterile H_2O
Sterigmatocystin (SMT)	po	DMSO
Cyclamate	po	H_2O
Saccharin	po	H_2O
2-Amino-3-methylimidazo[4,5-*f*]quinoline (IQ)	ng	HPC
2-Amino-3,8-dimethyimidazo[4,5-*f*]quinoxaline (MeIQx)	ng	HPC
2-Amino-1-methyl-6-phenylimidazo[4,5-*f*]pyridine (PhIP)	ng	HPC
Dichlorodiphenyltrichloroethane (DDT)	po	Corn oil
Arsenic	po	Sterile H_2O
Cigarette smoke condensate	Implant	Beeswax
Model rodent carcinogens		
Urethane	po, sc	Sterile H_2O
3-Methylcholanthrene (3-MC)	po, sc	Vitamin spread
3,4,9,10-Dibenzopyrene (BZP)	sc	Olive oil
2-Acetylaminofluorene (2-AAF)	po	Vitamin spread
2,7-Bisacetylaminofluorene (2,7-AAF)	po	Vitamin spread
3′-Methyl-4-dimethylaminoazo-benzene (3-MeDAB)	po	Vitamin spread
4-dimethylaminoazobenzene (Butter Yellow)	po	Apple sauce
Copper chelate of N-OH-AAF (CuCh)	sc	Sterile H_2O
N-Nitroso compounds		
N-Methyl-*N*-nitrosourea (MNU)	po	Karo syrup
N-Methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG)	po	Sterile H_2O
1-Nitrosopiperidine (PIP)	po, ip	Vitamin spread
Dimethylnitrosamine (DMNA)	ip	Sterile saline
Diethylnitrosamine (DENA)	po, ip	Sterile saline
Dipropylnitrosamine (DPNA)	ip	Sterile saline

[a] Some animals also received cycasin, methyl azoxymethanol-β-D-glucopyranoside, or crude cycad meal containing cycasin.

assigned to an untreated control group. For most compounds, exposure was initiated shortly after birth, at the time of weaning (approximately 6 months of age), or at 1 year of age. As a general rule, clinical doses were given for cancer therapeutic agents, but other compounds were administered at 10–40 times the estimated human exposure

level, or at maximally tolerated dose which did not cause organ toxicity or severe weight loss.

Each animal receives routine physical examination by a veterinarian every 6 months, and various hematological and biochemical tests are performed every 3 to 6 months, depending on the compound under study. For early detection of liver tumors, α-fetoprotein measurements, and abdominal laparoscopic examination are performed every 3 to 6 months, followed by wedge or needle biopsies taken from liver lesions for histopathological evaluation. Complete necropsies were performed on all animals that have died since the beginning of the carcinogenesis study.

RESULTS

The data on tumor incidence of animals surviving 6 months or longer after the first exposure to the test compounds is given. This eliminates animals that died as a result of acute toxicity during the first 6 months of dosing which was experienced with some of the earlier compounds. Since the latent period for tumor development for all of the compounds was more than 6 months, this would not distort the data on tumor incidence.

Spontaneous Tumors

A total of 373 autopsy records of breeders and normal controls were reviewed with respect to spontaneous tumors. As shown in Table 2, the incidence of malignant tumors was highest (8%) in the African green monkeys. Three of five malignancies in the African green monkeys were lymphomas (Table 3). The incidence of spontaneous malignant tumors was low in cynomolgus (1.5%) and rhesus (2.8%) monkeys (Table 2). These included two cynomolgus monkeys with adenocarcinoma of the kidney and the intestine, and five rhesus monkeys with sarcomas, squamous cell carcinomas, and adenocarcinoma (Table 3). Spontaneous benign tumors were detected in one cynomolgus and seven rhesus monkeys (Table 4).

Antineoplastic and Immunosuppressive Agents

Adriamycin. Initial dosing with Adriamycin was started in 1978. In the first group of 10 monkeys, dosing was started at 2 months of age and given monthly iv at 1.0

TABLE 2

SPONTANEOUS TUMOR RATE IN BREEDERS

Species	Total No. of monkeys	No. of monkeys with malignant tumors	No. of monkeys with benign tumors
Rhesus	181	5 (2.8%)	7 (3.9%)
Cynomolgus	130	2 (1.5%)	1 (0.8%)
African green	62	5 (8.0%)	0
Total	373	12	8

TABLE 3

SPONTANEOUS MALIGNANT TUMORS IN BREEDERS

Monkey number	Species	Sex	Date received	Observation period (months)[a]	Diagnosis
111K	Cyno	M	7/63	248	ACA, kidney
140T	Rhesus	F	7/64	140	Rhabdomyosarcoma
334G	Cyno	M	9/73	204	ACA, intestine
378T	Green	M	10/64	50	Lymphoma, histiocytic, poorly differentiated
379T	Green	F	10/64	120	Lymphoma, histiocytic, poorly differentiated
382T	Green	M	10/64	292	ACA, pancreas
413K	Rhesus	F	2/70	63	ACA, intrahepatic bile duct
492T	Rhesus	M	1/66	192	SCA, tongue
510T	Green	M	11/64	237	ACA, common bile duct
516T	Green	M	11/64	109	Lymphoma
577L	Rhesus	F	12/69	232	SCA, bladder
756L	Rhesus	F	2/70	247	Sarcoma, poorly differentiated

[a] From time of entry into colony until diagnosis of tumor. Abbreviations used: ACA, adenocarcinoma; CA, carcinoma; SCA, squamous cell carcinoma.

mg/kg for 30 months. A second group received 0.4 mg/kg once a month for 60 months, and the third group received 120 monthly doses of 0.2 mg/kg. Of the 30 monkeys dosed with Adriamycin, 2 have developed tumors thus far (Table 5). One rhesus monkey in the 1.0 mg/kg group developed acute nonlymphocytic leukemia (ANLL) after 32 months, and a cynomolgus monkey in the 0.4 mg/kg group was diagnosed

TABLE 4

SPONTANEOUS BENIGN TUMORS IN BREEDERS

Monkey number	Species	Sex	Date received	Observation period (months)[a]	Diagnosis
531	Rhesus	M	12/61	264	Papilloma of buccal mucosa and tongue; adenoma of adrenal cortex
132T	Rhesus	F	7/64	133	Papillary cystadenoma of ovary
140T	Rhesus	F	7/64	140	Islet cell adenomas of pancreas
158T	Rhesus	F	7/64	13	Hemangioma
329G	Rhesus	F	10/73	249	Leiomyoma of uterus
342G	Cyno	F	9/73	195	Hemangioma
589L	Rhesus	F	12/69	205	Ductal papilloma of breast
965L	Rhesus	F	12/69	61	Hemangioma

[a] From time of entry into colony until diagnosis of tumor.

TABLE 5

SUMMARY OF MALIGNANT TUMOR INCIDENCE IN MONKEYS DOSED WITH ANTINEOPLASTIC AND IMMUNOSUPPRESSIVE AGENTS

Agent	No. of monkeys[a]	Route	Dose (mg/kg)	No. alive	No. dead without malignant tumor	No. dead with malignant tumor
Adriamycin	10	iv	0.2	10	0	0
	10	iv	0.4	4	5	1
	10	iv	1.0	0	9	1
Cytoxan	23	po	6.0	15	7	1
Imuran	15	po	2.0	12	3	0
	14	po	5.0	12	2	0
L-PAM	20	ng	0.1	18	0	2
MIH	49	sc/po, ip/po, or ip	10	0	32	17
MNU	44	po	10	0	26	18

[a] Number of animals surviving >6 months after the first dose.

with low-grade fibrosarcoma 11 years after the first dose of Adriamycin. Interestingly, the fibrosarcoma was found in the popliteal region of the right leg, a site where Adriamycin had been injected iv. The development of Adriamycin-induced cardiac toxicity is well documented in humans (Bertazzoli *et al.*, 1971; Lindower and Skorton, 1992). Similarly, dose-dependent cardiomyopathy was observed in all of the monkeys in the 1.0 mg/kg group and five animals in the 0.4 mg/kg group which developed myocardial degeneration and died as a result of congestive heart failure (Sieber *et al.*, 1980b). The surviving monkeys do not show clinical evidence of congestive heart failure.

Cytoxan. Oral administration of Cytoxan to 23 monkeys was started in 1980, at a dose of 6.0 mg/kg 5 days per week. The 15 surviving monkeys in this group were taken off the compound after 11 years of dosing. Of the eight monkeys that have died in this group, one was found to have a small transitional cell carcinoma of the urinary bladder. Three monkeys had cardiac complications, including myocardial degeneration and fibrosis, chronic epicarditis, and chronic constrictive pericarditis. Three animals died from varicella infection during an outbreak in 1985, and one had no specific anatomic abnormalities at the time of death.

Imuran. Oral dosing of 29 monkeys with Imuran was started in 1977 at 2.0 and 5.0 mg/kg 5 days per week. Five animals have died in this group, but no tumors were detected. The dosing period was completed in 1992, and thus far, no tumors have been found in the surviving monkeys, all of which are in good health.

L-PAM (Melphalan). Dosing of 20 monkeys with L-PAM was started in 1975 and stopped in 1991. It was given po at 0.1 mg/kg five times per week. A higher incidence of endometriosis was observed in this group than in any of the other experimental groups. In 1988, two females were diagnosed with malignant tumors in the perineal and perivaginal areas. The tumors showed identical histological features, i.e., that of a poorly differentiated sarcoma of unknown origin. One of the animals had lung metastases at the time of necropsy. The association between the L-PAM treatment and the development of sarcomas is under consideration.

Procarbazine. Evaluation of the carcinogenic potential of procarbazine in the monkeys was initiated in 1963. Of 53 newborn monkeys that were placed on procarbazine, 49 survived 6 months or longer after the initial dose. All of the animals in this group are dead now, 17 (34.7%) of which were diagnosed with malignant tumors. The average cumulative dose in the tumor-bearing animals was 73.68 g (range, 2.69–177.63 g) and the average observation period was 119 months (range, 17–261 months). The malignant tumors included 8 ANLL, 1 lymphocytic lymphoma, 2 hemangiosarcomas, 3 osteosarcomas, 1 astrocytoma, and 1 colon carcinoma. One animal had two primary tumors, i.e., an islet cell carcinoma of the pancreas and a transitional cell carcinoma of the renal pelvis. The most common cause of death in the remainder of the group was bone marrow atrophy with lymphoid depletion. Another complication of procarbazine treatment was depression of spermatogenesis (Sertoli-only syndrome) which was observed in 18 of 29 male monkeys in the group (Sieber *et al.*, 1978).

N-Methyl-N-nitrosourea (MNU). Dosing of 44 monkeys with MNU was started in 1967 and was continued for 10 years (Adamson *et al.*, 1977). It was administered at 10 mg/kg in the formula for the first several months of life and was then incorporated into a vitamin-spread sandwich for the balance of the exposure period. The 18 animals that developed malignant tumors received an average dose of 140.42 g (range, 53.21–251.45 g) and the average latent period was 146.5 months (range, 62–264 months). Invasive squamous cell carcinoma of the esophagus was diagnosed in 15 monkeys (32%), 7 of which had a single primary tumor, and 8 had two or more tumors, most commonly located in the lower third of the esophagus. In addition, squamous cell carcinoma was detected in the oral cavity (7 cases), pharynx (3 cases), larynx (1 case), and stomach (2 cases). All except one of the cases occurred in animals with primary tumors at more than one site. One animal had three primary tumors, i.e., squamous cell carcinoma of the esophagus, adenocarcinoma of the stomach, and rhabdomyoblastoma of the heart. Another animal had leiomyosarcoma of the uterus and a squamous cell carcinoma of the esophagus, while the third animal had rhabdomyosarcoma at the base of the skull. All of the monkeys which developed squamous cell carcinoma of the esophagus also exhibited dysplasia of the nontumorous esophageal mucosa. Multifocal chronic esophagitis was seen in 26 cases, 13 of which had associated suprapapillary atrophy and 10 had chronic gastritis. Squamous papillomas of the esophagus were found in 5 cases and acanthosis in 5 cases.

Food Additives, Food Components, and Environmental Contaminants (Table 6)

Cyclamate. Administration of cyclamate was started in 1970, either at 100 or 500 mg/kg given po five times a week. The 500 mg/kg dose is equivalent to a daily intake of 11.6 g (about 30 diet drinks) for a 70-kg person. Of the 21 animals in this group, 7 have died so far (2 had severe endometriosis, 1 had chronic myocarditis, 1 had renal tubular degeneration, 2 died during the varicella outbreak, and 1 had no specific anatomic abnormalities). The 14 surviving monkeys are in generally good health considering their age. The cumulative doses range from 1.6 to 3.9 kg in the 100 mg/kg group and from 13.8 to 16.9 kg in the 500 mg/kg group.

In 1982, the metabolism of cyclamate in relation to testicular function was studied in 12 of the cyclamate monkeys and 3 controls after 12 years of chronic dosing. The following parameters were evaluated: testicular size and morphology, semen analysis,

TABLE 6

SUMMARY OF MALIGNANT TUMOR INCIDENCE IN MONKEYS DOSED WITH FOOD ADDITIVES, FOOD COMPONENTS, AND ENVIRONMENTAL CONTAMINANTS

Agent	No. monkeys[a]	Route	Dose (mg/kg)	No. alive	No. dead without malignant tumor	No. dead with malignant tumor
AFB_1	38	ip/po	0.2	0	15	23
Arsenic	20	po	0.1[b]	0	20	0
Butter yellow	7	po	120	0	6	1
3-Methyl-DAB	21	po	200[b]	0	20	1
Cigarette tobacco smoke condensate	10	imp	1.0[c]	0	10	0
Cycasin[d]	27	po	3.0	0	15	12
Cyclamate	10	po	100	8	2	0
	11	po	500	6	5	0
DDT	25	po	20	15	9	1
IQ	20	ng	10	10	1	9
	20	ng	20	3	0	17
MeIQx	10	ng	10	10	0	0
	10	ng	20	10	0	0
PhIP	10	ng	20	10	0	0
	10	ng	10	10	0	0
Saccharin	20	po	25	15	5	0
SMT	15	ng	1.0	7	3	5
	15	ng	2.0	1	9	5

[a] Number of animals surviving >6 months after the first dose.
[b] mg/day.
[c] ml/animal.
[d] Includes cycad meal and cycasin.

serum testosterone and gonadotropin levels, and relative cyclamate and cyclohexylamine levels excreted in urine. There were no detectable differences in testicular morphology, semen analysis, or hormone levels between the cyclamate monkeys and normal controls. The pattern of conversion of cyclamate to cyclohexylamine was comparable to that reported in humans (Renwick and Williams, 1972) with more than half of the animals either not converting or converting <1% of cyclamate to cyclohexylamine.

Saccharin. Dosing with sodium saccharin at 25 mg/kg 5 days a week was started in 1970. This dose corresponds to a daily intake of 5 cans of diet soda by a 70-kg person. Of 20 monkeys on study, 5 have died (2 died during the varicella outbreak, 1 died of bronchopneumonia, and 2 had no specific anatomic abnormalities). Tumors have not been detected in any of the dead animals and none of the 15 surviving monkeys show evidence of tumor development or other signs of illness.

Aflatoxin B_1 (AFB_1). Carcinogenicity studies of AFB_1 in nonhuman primates were started in 1966. Oral dosing was started at 2 weeks of age. For the first month it was given two to three times weekly at 0.05 mg/kg. The dose was raised steadily over the next 6 months, up to 0.8 mg/kg, using the same dosing frequency. A second group of

newborn monkeys received AFB_1 ip, starting at 0.2 mg/kg once weekly, and reaching 0.25 mg/kg at 2 months of age. The third group was dosed both orally and ip. In the first group, 28 animals survived more than 6 months, 18 (64.3%) of which developed malignant tumors. In the second and third groups, 5 animals survived more than 6 months in each group, of which 5 (50%) developed malignant tumors. Dosing of AFB_1 was continued until diagnosis of malignancy was established or until the end of an observation period of 18 years. A third of the monkeys with malignancies had more than one primary tumor. A total of 29 tumors were found in 23 animals which included 10 bile duct carcinomas, 3 hepatocellular carcinomas, 3 adenocarcinomas of the pancreas, 7 hemangiosarcomas of the liver, 3 osteosarcomas, 2 transitional cell carcinomas of the urinary bladder, and 1 fibrosarcoma in the pelvic area (Adamson *et al.*, 1976). The average cumulative dose for the tumor-bearing animals was 884.84 mg (range, 99.19–1609.06 mg) and the average observation period was 139.2 months (range, 48–210 months). Tumor incidence was considerably higher in females (16 of 23, 70%) than in males (7 of 16, 44%). Among the 15 animals without tumors, 6 died as a result of toxic hepatitis, 4 developed cirrhosis, and 3 had hyperplastic nodules in the liver at the time of death.

Cycasin, MAM-acetate. A group of 27 animals received either cycasin (50–75 mg/kg, orally, 5 times/week) or its aglycone, MAM-acetate (1.5–3.0 mg/kg, po and ip, 5 times/week) for approximately 18 years. Of this group, 12 (44.4%) developed one or more malignancies. These included 7 hepatocellular carcinomas, 2 bile duct carcinomas, 4 renal cell carcinomas, 2 squamous cell carcinomas of the esophagus, 1 adenocarcinoma of the pancreas, 1 adenocarcinoma of the small intestine, 1 colon carcinoma, 1 hemangiosarcoma, and 1 cholangiosarcoma. The latent periods ranged from 7 to 24 years. Many of the animals without tumors died as a result of toxic hepatitis and cirrhosis (Sieber *et al.*, 1980a).

Sterigmatocystin (SMT). In 1975, 30 young adult monkeys were placed on SMT at 1.0 and 2.0 mg/kg po once a week. So far, a total of 10 SMT monkeys have developed one or more malignant tumors, 5 in each dose group. These include 7 hepatocellular carcinomas, 2 cholangiocarcinomas, 1 cholangiosarcoma, and 1 renal cell carcinoma. The average cumulative dose in the tumor-bearing monkeys in the 1.0 mg/kg group was 1.96 g (range, 1.12–3.39 g) and the average latent period was 147 months (range, 74–182 months). In the 2.0 mg/kg group, the average cumulative dose was 3.97 g (range, 2.57–5.00 g), and the average latent period was 138 months (range, 101–161 months). The diagnoses in monkeys that died without tumors included myocarditis, sepsis, toxic hepatitis, cirrhosis, bronchopneumonia, acute renal tubular necrosis, and necrotizing colitis. In the surviving monkeys that are still receiving SMT, there is evidence of extensive liver damage as visualized by laparoscopy.

Heterocyclic aromatic amines (HAA). The three HAA under evaluation include 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (8-MeIQx), and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP). Dosing of monkeys with IQ was started in 1985, 8-MeIQx in 1987, and PhIP in 1990. All three compounds are administered through nasogastric intubation at 10 and 20 mg/kg, 5 days a week. Thus far, IQ has proven to be a potent carcinogen in the monkeys. Malignant liver tumors have been found in 17 of 20 animals in the 20 mg/kg group and in 9 of 20 in the 10 mg/kg group (Adamson, 1989; Adamson *et al.*, 1990, 1991). The first case of metastatic hepatocellular carcinoma was diagnosed after

27 months of dosing with IQ. The monkeys dosed with 8-MeIQx and PhIP at present show no signs of liver abnormalities or other pathological findings.

DDT. A group of 15 monkeys was started on DDT in 1969 and another group of 10 monkeys in 1975. DDT was administered to both groups at 20 mg/kg po 5 times per week for a period of 130 months. To date, 10 animals in this group have died. One developed hepatocellular carcinoma after an observation period of almost 20 years and a total dose of 292 g. There was clinical evidence of CNS toxicity in five animals which experienced severe tremors and convulsions immediately prior to death. One animal died as a result of toxic hepatitis $4\frac{1}{2}$ years after the first dose of DDT, and two animals developed diabetes after 14 and 17 years. One animal was diagnosed with Sertoli-only syndrome (Sieber *et al.*, 1978). The 15 surviving animals are all in good health 16–22 years after DDT was first administered. It is unclear whether the single case of hepatocellular carcinoma with such long latent period is associated with exposure to DDT.

Arsenic. Dosing of 20 cynomolgus feral monkeys with arsenic was initiated in 1976. It was administered po as sodium arsenate at 0.1 mg/kg, 5 days/week. In 1991, when dosing of arsenic was stopped, the study was terminated and the 11 surviving animals were euthanized. Cortical adenomas were found in the kidneys of 2 of the 11 animals. Other notable findings included 1 case of micronodular cirrhosis, 3 cases of endometriosis, and 3 cases of hyalinized Langerhans islets of the pancreas, one of which showed clinical evidence of diabetes. None of the 20 animals in this group developed malignant tumors.

Butter yellow. Butter yellow was among the first compounds to be evaluated in the monkey colony in 1961 and 1962. It was administered po at 120 mg/kg from birth to 5 years of age. Seven of 11 animals survived more than 6 months after the first dose of butter yellow. Of the 7 animals, 4 developed liver degeneration within 2 years and a cumulative dose range of 13.11–17.44 g. One animal died after approximately 5 years from toxic hepatitis and a cumulative dose of 102.8 g. One had no specific anatomic abnormalities at the time of autopsy. The last animal in this group was found to have bronchoalveolar carcinoma after an observation period of almost 20 years and 136.2 g of the compound. Since this carcinoma occurred in an aged animal, it is not possible to determine if it was induced by butter yellow.

3'-Methyl-4-dimethylaminoazo-benzene (3-methyl-DAB). A total of 21 animals received increasing doses of 3-methyl-DAB (10–200 mg/day), 3–5 times weekly for 5 years. All of the animals in this group have been autopsied. Toxic hepatitis and liver necrosis was the cause of death in 6 of the animals. Hepatocellular carcinoma was diagnosed in one animal after an observation period of almost 20 years and a cumulative dose of 191.85 g. Hemangiomas of the liver and skin were detected in another animal after 24 years and 183.50 g of the compound. The rest of the animals in this group died from miscellaneous causes. As with butter yellow, the carcinoma found in the aged 3-methyl-DAB monkey may or may not be compound-related.

Cigarette smoke condensate and beeswax. Lung implants containing cigarette smoke condensate (1 mg) in a beeswax matrix were introduced into the subpleural space of two groups of 10 monkeys, and 6 animals received the beeswax alone. After a 20-year observation period all of the animals were euthanized. No tumors were found, but foreign body granulomas were seen in 3 of the cases.

"Classical" rodent carcinogens (Table 7). These agents were among the first to be tested in the monkey colony, starting in 1962. They included 2-acetylaminofluorene

TABLE 7

SUMMARY OF MALIGNANT TUMOR INCIDENCE IN MONKEYS DOSED WITH CLASSICAL RODENT CARCINOGENS

Agent	No. monkeys[a]	Route	Dose (mg/kg)	No. alive	No. dead without malignant tumor	No. dead with malignant tumor
2-AAF	13	po	120	0	12	1
2,7-AAF	14	po	100	2	11	1
BZP	17	sc	10–20	0	17	0
	10	imp	120[b]	0	10	0
CuCH of N-OH-AAF	9	sc	20–30	0	9	0
3-MC	18	po	120	0	18	0
		sc	10–40			
Urethane	32	po	250	0	26	6

[a] Number of animals surviving >6 months after the first dose.
[b] mg/day.

(2-AAF), 2,7-AAF, the copper chelate of *N*-hydroxy-AAF (CuCH), 3,4,9,10-dibenzopyrene (BZP), 3-methylcholanthrene (3-MC), and urethane. The animals were dosed for 5 years at levels comparable to those given to rodents. Interestingly, only urethane turned out to be an unequivocal carcinogen which was dosed at 250 mg/kg, po 5 days a week for 5 years. Some of the urethane monkeys also received 7–10 weekly courses of whole body radiation (50 rad per course). Six of 32 monkeys dosed with urethane developed one or more primary tumors. These included adenocarcinomas of lung, pancreas, bile ducts and small intestine, hepatocellular carcinoma, hemangiosarcoma and adenoma of the liver, ependymoma, pheochromocytoma, endocervical adenofibroma, and squamous papilloma of the pouch. The average cumulative dose for the tumor bearing animals was 235 g (range, 214.82–339.25 g) and the average latent period was 186 months (range, 145–267 months). Only 2 of the 6 monkeys with malignant tumors had been irradiated, indicating that urethane is carcinogenic in monkeys regardless of subsequent radiation. The urethane monkeys that did not develop tumors died of miscellaneous causes which did not seem to be compound related.

In the 2-AAF group of 13 monkeys, 10 survived for 20 years or longer, one of which developed adenocarcinoma of the nipple. The average cumulative dose in the group without tumors was 139.9 g (range, 102.0–158.9 g) and the average observation period was 240 months (range, 14–318 months). For the animal with the breast carcinoma, the cumulative dose was 146.1 g and observation period was 268 months. One of 14 monkeys in the 2,7-AAF group developed medullary carcinoma of the breast with a cumulative dose of 105.8 g and a latent period of 311 months. The average cumulative dose was 97.8 g (range, 56.4–107.0 g) and the average observation period was 202 months (range, 30–319 months). Two animals in this group are still alive. No tumors were detected in the groups that received CuCh, BZP, or 3-MC. In the CuCh group, the average cumulative dose was 0.21 g (range, 0.13–0.24 g) and the average observation period was 252 months (range, 3–353 months). In the BZP group given sc injections, the average cumulative dose was 139.5 g (range, 12–460 g) and the average observation period was 222 months (range, 19–324 months). BZP (32 mg per animal) was also

TABLE 8

SUMMARY OF MALIGNANT TUMOR INCIDENCE IN MONKEYS DOSED WITH NITROSO COMPOUNDS

Agent	No. monkeys[a]	Route	Dose (mg/kg)	No. alive	No. dead animals without tumor	No. dead animals with tumor
DENA	40	po	10 → 40	0	9	31
DENA	128	ip	10 → 40	0	16	112
DENA (galagos)	14	ip	30.0	0	4	10
DPNA	6	ip	40.0	0	0	6
PIP	10	ip	40.0	0	4	6
	12	po	400.0	0	1	11
MNNG	20	po	4.0	0	20	0
	3	imp[b]	4.0	0	2	1
DMNA	7	ip	7.25	0	7	0

[a] Number of animals surviving >6 months after the first dose.
[b] Inserted intrarectally.

given as lung implants to 10 monkeys as described above for cigarette smoke condensate. No tumors were found after a 20-year observation period. In the 3-MC group, the average cumulative dose was 73.0 mg (range, 0.06–117.25 mg) and the average observation period was 191 months (range, 9–331 months).

N-Nitroso compounds (Tables 8 and 9). *N*-Nitroso compounds were first tested in the monkey colony in 1962. These included diethylnitrosamine (DENA), dimethylnitrosamine (DMNA), dipropylnitrosamine (DPNA), *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), and 1-nitrosopiperidine (PIP).

All of the compounds, except DMNA and MNNG, were shown to be hepatocarcinogens in the monkeys; DMNA, however, was a potent liver toxin.

DENA. DENA has proven to be the most potent and predictable hepatocarcinogen of all agents tested in this study. Tumors were induced after po and ip administration, but the ip route has resulted in the shortest latent period (minimum of 6 months), and an incidence of close to 100%. A study was initiated comparing DENA-mediated tumor induction in newborn rhesus, cynomolgus, African green, and rhesus–cyno-

TABLE 9

TUMOR INCIDENCE, AVERAGE CUMULATIVE DOSE, AND LATENT PERIOD IN MONKEYS DOSED ip WITH DENA

Dose of DENA (mg/kg ip)	No. monkeys	No. monkeys with HCA	Average cumulative dose (range) (g)	Average latent period (range) (months)
0.1	11	0	0.104 (0.056–0.146)	—
1	11	4	1.78 (1.66–2.74)	157 (111–177)
5	10	10	2.70 (1.47–5.81)	74 (52–109)
10	11	11	2.02 (1.22–4.14)	38 (14–64)
20	11	11	2.40 (0.83–4.65)	25 (13–39)
40	106	106	1.59 (0.39–4.08)	16 (6–49)

molgus hybrids. DENA was given ip at a gradually increasing dose, from 10 to 40 mg/kg every 14 days during the first 6 months of life, and dosing was continued until tumor was detected. There were minor differences in the cumulative dose and latent period between the three species.

DENA was used as a model hepatocarcinogen to study the relationship between cumulative dose and latent period for tumor induction. DENA was administered ip at doses of 0.1, 1, 5, 10, 20, and 40 mg/kg. In the 5, 10, 20, and 40 mg/kg groups all of the animals developed hepatocellular carcinomas (Table 9). In the 1 mg/kg group, 4 of 11 animals have developed tumors so far, but none in the 0.1 mg/kg group. The average cumulative dose was lowest (1.59 g) in the 40 mg/kg group, but in the other groups it was comparable. The total carcinogenic dose of DENA was calculated as 2.18 g from the average cumulative dose values from the 5, 10, 20, and 40 mg/kg groups. The animals at 0.1 mg/kg have been dosed for 10 years, and it is unlikely that they will survive long enough to receive cumulative doses of 2.18 g. When the dose of DENA was plotted against the average latent period, there was a linear relationship between the three highest doses, as shown in Fig. 1. However, at 5 mg/kg a marked prolongation of the latent period was observed.

DENA was given ip at 30 mg/kg to 14 prosimian bushbabies (*Galago crassicaudatus*), 10 of which developed mucoepidermoid carcinoma of the nasal cavity (Table 8). Two of the 10 bushbabies also developed hepatocellular carcinoma. The average cumulative dose was 0.75 g (range, 0.30–1.49 g) and the latent period was 22.9 months (range, 15–32 months).

MNNG and DMNA. Among 20 monkeys dosed with MNNG po at 4 mg/kg, the average cumulative dose was 34.8 g (range, 1.7–56.2 g), and the average observation period was 206 months (range, 71–238 months). None of the animals developed malignant tumors, but a liver adenoma was incidentally found in one of the longest survivors. Another animal was diagnosed with hyperplastic nodules of the liver after an observation period of 92 months.

A pilot study was undertaken where MNNG was inserted in gelatin into the rectum of three monkeys. Of these, one animal developed an adenocarcinoma of the rectosigmoid junction after a total dose of 8.5 g and a latent period of 73 months.

DMNA was given ip at 10 mg/kg every 2 weeks to 11 monkeys. Of 7 animals that survived longer than 6 months, one died of cirrhoses after 8 months, and the 6 remaining animals died as a result of toxic hepatitis. The average cumulative dose was 7 g (range, 0.06–12.35 g) and the average observation period was 54 months (range, 8–118 months).

DPNA. All of the 6 monkeys in this group developed hepatocellular carcinoma. The animals received 11 injections every 2 weeks, ranging from 13 to 40 mg/kg. The average cumulative dose was 6.97 g (range, 6.07–7.86 g), and the average latent period was 29 months (range, 22–33 months).

PIP. The 22 animals in this group were dosed either po (12 animals) or ip (10 animals). The daily po dose was started at 10 mg/kg and increased to 400 mg/kg and the ip dosing schedule was the same at 10 times lower dose. The average cumulative dose for tumor development was much lower when PIP was administered by an ip route. In the 6 tumor-bearing animals dosed ip the average cumulative dose was 42.95 g (range, 26.69–51.47 g) and in the 11 tumor-bearing animals dosed po was 1625.91 g (range, 177.02–2662.32 g). The reverse was observed regarding the average latent period, which was 141 months (range 79–239 months) for the ip group and 87 months

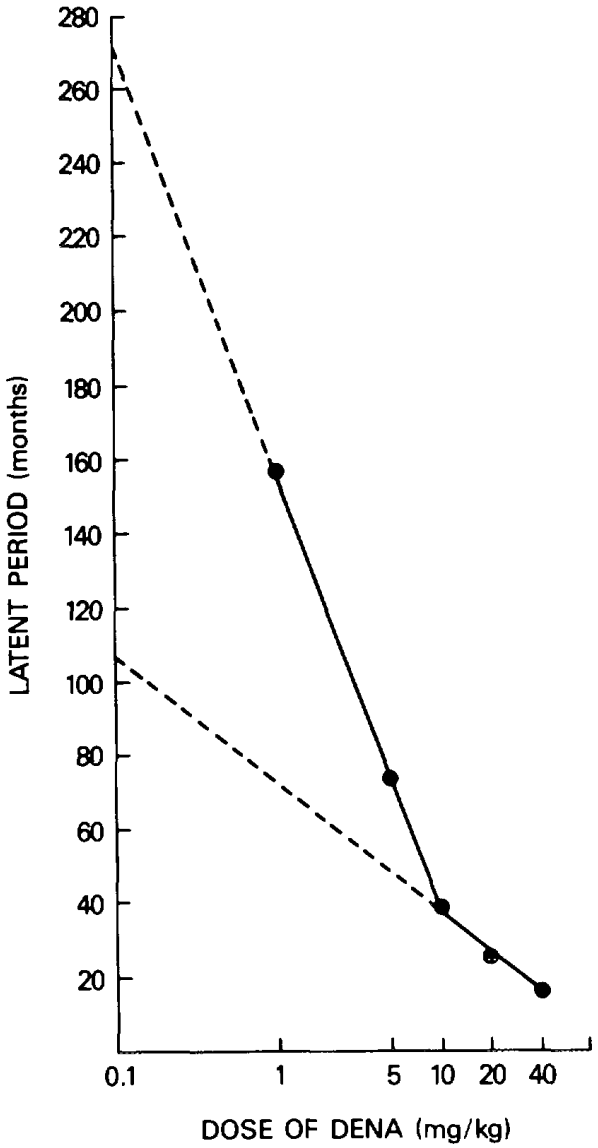

FIG. 1. Semilog plot of the DENA dose (milligram/kilogram) against the average latent period for tumor induction in monkeys dosed ip at 0.1, 1, 5, 10, 20, and 40 mg/kg.

(range, 36–145 months) after dosing po. The five animals that did not develop tumors died as a result of toxic hepatitis within 6–7 years; four of those were dosed ip and one po.

DISCUSSION

The choice of models for chemical carcinogenesis bioassays is usually based on the availability and cost of experimental animals, rather than similarities of metabolic pathways to humans. Consequently, carcinogenic potencies for chemicals have mainly been determined in rats and mice. Extrapolation of data from long-term rodent bioas-

says to humans has created problems due to the high incidence of spontaneous tumors, differences in drug metabolism, life span differences, and reproductive differences in these animals (Roe, 1987). In addition, there are questions relative to the high doses used in rodent bioassays and the relevance of these doses to human exposures. Although it has been argued that carcinogenesis data from nonhuman primates are more meaningful than rodent data, it can also be debated how accurately they reflect human cancer risk. Earlier studies on comparative drug metabolism in different species revealed that metabolic pathways, such as N-glucuronidation and O-methylation were more highly developed in primates than in nonprimate species (Smith and Williams, 1974; Dawn and Chasseaud, 1981). However, there is a need to study more closely in nonhuman primates the drug metabolizing enzymes, such as the different isoforms of cytochrome P450, which are involved in metabolic activation of many of the compounds being evaluated in this study (Davis *et al.*, 1993). Regardless of the similarities and differences in metabolic pathways to humans, nonhuman primates represent a highly valuable experimental animal model for studies of the carcinogenic process. Due to the longevity of Old World monkeys used in this study (25–30 years), chronic exposure for many years is more comparable to human experience. Furthermore, the relatively large size of the monkey organs makes it feasible to perform diagnostic procedures repeatedly on the same animal over a long period. Another significant advantage for using nonhuman primates in carcinogenesis studies is the low spontaneous tumor rate (Lingeman *et al.*, 1968; O'Gara and Adamson, 1972). In reviewing autopsy reports of 373 breeders and untreated controls, the spontaneous malignant tumor rate for cynomolgus monkeys was 1.5% and for rhesus monkeys 2.8%. In African green monkeys the spontaneous tumor rate was 8%, which is closer to that observed in rodents. Two of the spontaneous malignancies in the African green monkeys were histiocytic lymphomas (O'Gara *et al.*, 1971).

Some of the commonly used antineoplastic and immunosuppressive agents have been found to be carcinogenic in rodents (Weisburger, 1977). Of the six agents evaluated in this category, two have clearly proved to be carcinogenic, i.e., procarbazine and MNU in the monkeys. Procarbazine (MIH) was used as a single antitumor agent for the treatment of Hodgkin's disease prior to the development of the MOPP (mechlorethamine, vincristine, procarbazine, prednisone) regimen, which has greatly prolonged the survival of patients with Hodgkin's disease (DeVita *et al.*, 1970). An increased incidence of leukemia was observed in long-term survivors of Hodgkin's disease following treatment with the MOPP regimen (Rosner and Grunwald, 1975; Adamson and Sieber, 1977), but it has not been possible to determine whether procarbazine is responsible for the second malignancy. ANLL was found in 8 of 17 monkeys with malignancies in the procarbazine group, and a third of the animals, which did not develop tumors, died as a result of myelosuppression (Sieber *et al.*, 1978). This finding has also been reported in patients treated with procarbazine (Reed, 1975).

N-Methyl-*N*-nitrosourea (MNU) is a direct-acting carcinogen which does not require enzymatic activation. Various types of tumors have been documented in experimental animals following exposure to MNU (Swenberg *et al.*, 1972; Schreiber *et al.*, 1969; Dimant and Beniashvili, 1978; Denlinger *et al.*, 1978; Narisawa *et al.*, 1976). Hormone responsive mammary carcinomas were induced at a high rate in rats following iv injection of MNU (Gullino *et al.*, 1975). Antitumor activity of MNU has been demonstrated in experimental animal models (Sugiura, 1967) and it was used in the Soviet Union for treating Hodgkin's disease and undifferentiated carcinoma of the lung

(Emanuel *et al.*, 1974). Other nitrosoureas, i.e., *N,N'*-bis(2-chloroethyl)-*N*-nitrosourea (BCNU), *N*-(2-chloroethyl)-*N'*-cyclohexyl-*N*-nitrosourea (CCNU), and methyl-CCNU, which are used in the treatment of a variety of human tumors, have been associated with increased risk of acute leukemias (Cohen *et al.*, 1976). MNU was the only compound in this carcinogenesis study that produced malignancies of the digestive tract in significant numbers. It induced squamous cell carcinoma of the pharynx, larynx, and esophagus in more than one-third of the animals. Since MNU does not require metabolic activation for its carcinogenic effect, one can assume that it acted directly on the digestive tract following po administration to the monkeys. The carcinoma was frequently associated with other lesions in the esophagus, such as chronic esophagitis, multifocal atrophy, acanthosis, and dysplasia. As has been proposed in humans, these lesions may precede invasive carcinoma (Correa, 1982).

L-PAM treatment has been associated with increased incidence of acute leukemias in patients with multiple myeloma (Karchmer *et al.*, 1974; Kyle *et al.*, 1974). In a group of 20 monkeys dosed with L-PAM for 16 years, two developed soft tissue sarcoma. Considering that both cases of sarcoma were of the same histological type and occurred at a similar location, it is likely that they are related to L-PAM exposure, although the surviving animals show no evidence of tumor formation.

Adriamycin (doxorubicin) is an anthracycline with a wide spectrum of antitumor activity (Arcamone, 1981). It has been shown to be mutagenic in the Ames test and carcinogenic in rodents (Bertazzoli *et al.*, 1971). In patients, the most serious complication of Adriamycin treatment is its cardiotoxicity (Lanaz and Page, 1976). Similarly, Adriamycin had serious cardiotoxic effects in the monkeys dosed at 1 mg/kg, where all of the animals died as a result of congestive heart failure (Sieber *et al.*, 1980b). The association of Adriamycin with the two tumor cases detected so far in this group is not clear, although one of the tumors, i.e., a low-grade fibrosarcoma, was found in the region where Adriamycin had been injected iv 11 years earlier.

Cytoxan (cyclophosphamide) is used for treating many types of cancer. Both cytoxan and the immunosuppressive agent, Imuran, have also been used for prolonged treatment of rheumatoid arthritis. Long-term treatment with both drugs has been connected to different types of malignancies, such as leukemias and bladder cancer (Scharf *et al.*, 1977; Fairchild *et al.*, 1979; Sheibani *et al.*, 1980; Schmahl *et al.*, 1982). A 20-year follow-up study was published on the incidence of lymphoma in patients with rheumatoid arthritis treated with Imuran (Silman *et al.*, 1988). A 10-fold increase in the incidence of lymphoma was found in the Imuran-treated group over the general population and a 5-fold increase was observed in a control group of patients with rheumatoid arthritis matched for age and year of diagnosis. The dose of Imuran and the length of the dosing period were comparable to those used for the monkeys in this study that have so far not revealed any signs of tumor development or other types of ailment. Similarly, cytoxan thus far has not been found to be highly carcinogenic in the monkeys, although one monkey had a carcinoma of the urinary bladder.

With the exception of the heterocyclic aromatic amines (HAA), the compounds listed as food additives, food components, and environmental contaminants have been under observation for many years, and in some cases, for the entire life span of the animals. AFB_1, SMT, and cycasin were clearly carcinogenic, inducing mostly cancers of hepatocellular origin in significant proportion of the animals. However, in the animals that received DDT, butter yellow, or 3-methyl-DAB, only one malignant tumor was found in each group. In the DDT group, 14 animals are still alive and have

been dosed continuously for up to 22 years. The carcinogenicity of DDT is well established in rodents (Innes *et al.*, 1969). However, it remains to be determined whether the single case of hepatocellular carcinoma detected so far was induced by DDT, or if it represents a spontaneous tumor in an aged monkey. Arsenic represents another important compound among the environmental contaminants. It has been suggested that chronic exposure to inorganic arsenic can cause skin and lung cancer (*IARC Monograph*, 1980). A recent study on the cancer risk estimate from arsenic in drinking water presented evidence of a causal relationship between ingested arsenic and cancer of the liver, lung, kidney, and bladder (Smith *et al.*, 1992). Arsenic did not prove to be carcinogenic in the monkeys that were dosed continuously for 17 years. When the study was terminated in 1991, all of the arsenic monkeys were euthanized and necropsied. Renal cortical adenomas were found in two of the animals, but no malignant tumors were detected.

The chemicals in this category most recently introduced in the monkey colony are heterocyclic aromatic amines (HAA), which are formed during cooking of meat (Ito *et al.*, 1991). Mutagenic HAA fall into two classes, i.e., an imidazole class with an amino group attached to the 2-position of an imidazole ring, and a nonimidazole class with an amino group attached to a pyridine ring (Sugimura *et al.*, 1977; Commoner *et al.*, 1978; Felton and Knize, 1991). The following three HAA are under evaluation in this study: 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (8-MeIQx), and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP). These agents are found in meat cooked by frying, broiling, or grilling. One gram of fried ground beef contains 0.02–0.6 ng of IQ, 0.1–2.4 ng of 8-MeIQx, and 15 ng of PhIP. The mutagenic activity in the Ames test was found to be much weaker for PhIP than for IQ and MeIQx. All three compounds are carcinogenic in mice and rats, producing tumors in different organ systems (Ohgaki *et al.*, 1991; Ito *et al.*, 1991). Since dosing of IQ was started in 1985, 17 of 20 animals receiving 20 mg/kg and 9 of 20 receiving 10 mg/kg, have developed hepatocellular carcinomas. Considering the short latent period and extensive hepatic tumor involvement, this compound has turned out to be, next to DENA, the most potent hepatocarcinogen in the monkey colony. Current studies on this group, which consists mostly of cynomolgus monkeys, involve metabolic activation and processing of IQ, DNA adduct measurements, and characterization of IQ-induced liver tumors (Snyderwine *et al.*, 1988; Adamson *et al.*, 1991).

Extensive rodent bioassays and epidemiological studies have been carried out on the artificial sweeteners, cyclamate and saccharin. Cyclamate was banned in the United States and a number of other countries following a report on the induction of urinary bladder tumors in rats fed high doses of a cyclamate:saccharin mixture, or the metabolite of cyclamate, cyclohexylamine (Price *et al.*, 1970). In contrast to the findings in rodents, none of the monkeys in this study, exposed to high doses of cyclamate and saccharin since 1970, have shown any evidence of tumor development. In this regard, the lack of carcinogenicity in monkeys is more consistent with recent evidence from epidemiological studies suggesting that individuals consuming cyclamate and/or saccharin regularly are not at increased risk of developing cancer (Walker *et al.*, 1982; Chappel, 1992). A wide variation in the metabolic activity of cyclamate has been reported both between individuals and in the same individual (for review, see Renwick, 1986). The rate and extent of the conversion of cyclamate to cyclohexylamine has been associated with irreversible testicular atrophy in rats (Mason and Thompson,

1977). When the cyclamate monkeys were evaluated after 12 years of chronic dosing, no abnormalities in testicular morphology and function were detected (unpublished observations). In the groups of monkeys chronically dosed with cyclamate and saccharin, the oldest animals are approaching the maximum life span for cynomolgus and rhesus monkeys of 25–30 years. It is therefore likely that this study on cyclamate and saccharin will eventually represent a lifetime observation of their toxic and carcinogenic potential.

AFB_1, produced by *Aspergillus flavus,* is carcinogenic in a wide variety of animal models (*IARC Monograph,* 1971). In this study, it was an unequivocal carcinogen, inducing primarily carcinomas of hepatobiliary origin (Sieber *et al.,* 1979). Epidemiological studies suggest that AFB_1 represents an important risk factor for liver cancer in certain geographic areas of Africa and China (Sun *et al.,* 1986). AFB_1 may also pose a carcinogenic risk in the Western World according to a recent study from Great Britain (Harrison *et al.,* 1993). Studies on cancer-related genetic changes have focused on the mutational spectrum of the p53 tumor suppressor gene. In the Qidong region of China and southern Africa, where AFB_1 and hepatitis B virus are considered important risk factors for liver cancer, specific G to T mutation in codon 249 of the p53 gene were observed in almost half of the hepatocellular carcinomas studied (Hsu *et al.,* 1991; Bressac *et al.,* 1991). These findings prompted a study on p53 mutations in the malignant liver tumors induced by AFB_1 in the monkeys. None of the monkey liver tumors possessed the mutational spectrum (Fujimoto *et al.,* 1992) described above which in human liver cancer has been associated with AFB_1 exposure (Hollstein *et al.,* 1991). The second fungal metabolite tested in the monkey was SMT which is structurally and biochemically related to AFB_1 (*IARC Monograph,* 1971). It is hepatotoxic and carcinogenic in rodents (Ohtsubo *et al.,* 1978) and, like AFB_1, has been associated with the high incidence of liver cancer in Africa. As was observed for AFB_1, SMT has proven to be carcinogenic in the monkeys with the development of malignant liver tumors in one-third of the animals.

Interestingly, only one of the classical rodent carcinogens, i.e., urethane, was clearly carcinogenic in the monkey colony. Animals dosed with 2-AAF, BZP, and 3-MC were under observation for up to 25 years. The two animals that developed malignancies were a rhesus monkey in the 2-AAF group, which was diagnosed with a small adenocarcinoma of the nipple at age 24, and a cynomolgus monkey in the 2,7-AAF group with a medullary carcinoma of the breast and a bronchioalveolar carcinoma at age 25. It is likely that tumor development in these animals is more closely associated with old age than compound related.

N-Nitroso compounds induce a variety of tumors in a range of laboratory animals (*IARC Monograph,* 1971; Bogovski and Bogovski, 1981). Of the five *N*-nitroso compounds tested in the monkeys, three were carcinogenic, i.e., DENA, DPNA, and PIP. DENA, which is a highly potent rodent hepatocarcinogen (Cortinovis *et al.,* 1991) also turned out to be a powerful hepatocarcinogen in rhesus, cynomolgus, and African green monkeys with a latent period as low as 6 months, when administered ip at 40 mg/kg. In a group of 14 bushbabies dosed ip with DENA, the compound induced mucoepidermoid carcinoma of the nasal cavity in 10 of the cases with associated hepatocellular carcinoma in 2 of the animals. DENA was used as a model hepatocarcinogen to assess the relationship between the cumulative dose and the latent period for tumor induction. As shown in Fig. 1, there was a linear relationship between the DENA dose and the latent period for the 40, 20, and 10 mg/kg groups, and also for

the 10, 5 and 1 mg/kg groups. If the linear relationship will be maintained, the latent period for the 0.1 mg/kg group (272 months) will be approaching their expected life span of approximately 25–30 years. The average cumulative dose for tumor development was 2.18 g in the 40, 20, 10, and 5 mg/kg groups. Taking this as the carcinogenic dose for DENA, the average cumulative dose of 1.8 g for the 1 mg/kg group will approach the carcinogenic dose after 13 years.

This chemical carcinogenesis study in nonhuman primates, which has been in existence since 1961, has generated a wealth of valuable data on a variety of potential human carcinogens. Of particular interest to human risk assessment are the recent data on the carcinogenic effects of the heterocyclic aromatic amine IQ, which is formed during cooking of meat (Adamson *et al.*, 1990, 1991). The lack of toxicity and carcinogenicity of cyclamate and saccharin following ingestion of high doses for 22 years may also be of major significance. When studies on these compounds are completed, the data should be useful in human risk assessment.

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